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(54) Title: NOVEL PROKARYOTIC POLYNUCLEOTIDES, POLYPEPTIDES AND THEIR USES			
(57) Abstract The invention provides novel polypeptides and polynucleotides encoding such polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing such polypeptides to screen for antibacterial compounds.			

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**NOVEL PROKARYOTIC POLYNUCLEOTIDES,
POLYPEPTIDES AND THEIR USES**

RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application Number 60/024,022,
5 filed August 16, 1996.

FIELD OF THE INVENTION

This invention relates to newly identified polynucleotides and polypeptides, and their
production and uses, as well as their variants, agonists and antagonists, and their uses. In
particular, in these and in other regards, the invention relates to novel polynucleotides and
10 polypeptides set forth in the Sequence Listing.

BACKGROUND OF THE INVENTION

The Streptococci make up a medically important genera of microbes known to cause
several types of disease in humans, including, for example, otitis media, conjunctivitis,
pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most
15 particularly meningitis, such as for example infection of cerebrospinal fluid. Since its isolation
more than 100 years ago, *Streptococcus pneumoniae* (*S. pneumoniae*) has been one of the more
intensively studied microbes. For example, much of our early understanding that DNA is, in
fact, the genetic material was predicated on the work of Griffith and of Avery, Macleod and
McCarty using this microbe. Despite the vast amount of research with *S. pneumoniae*, many
20 questions concerning the virulence of this microbe remain. It is particularly preferred to employ
Streptococcal genes and gene products as targets for the development of antibiotics.

The frequency of *Streptococcus pneumoniae* infections has risen dramatically in the past
20 years. This has been attributed to the emergence of multiply antibiotic resistant strains and an
increasing population of people with weakened immune systems. It is no longer uncommon to
25 isolate *Streptococcus pneumoniae* strains which are resistant to some or all of the standard
antibiotics. This has created a demand for both new anti-microbial agents and diagnostic tests
for this organism.

Clearly, there is a need for factors, such as the novel compounds of the invention, that
have a present benefit of being useful to screen compounds for antibiotic activity. Such factors
30 are also useful to determine their role in pathogenesis of infection, dysfunction and disease.
There is also a need for identification and characterization of such factors and their antagonists
and agonists which can play a role in preventing, ameliorating or correcting infections,
dysfunctions or diseases.

The polypeptides of the invention have amino acid sequence homology to a known protein(s) as set forth in Table 1.

SUMMARY OF THE INVENTION

It is an object of the invention to provide polypeptides that have been identified as novel polypeptides by homology between an amino acid sequence selected from the group consisting of the sequences set out in the Sequence Listing and a known amino acid sequence or sequences of other proteins such as the protein identities listed in Table 1.

It is a further object of the invention to provide polynucleotides that encode novel polypeptides, particularly polynucleotides that encode polypeptides of *Streptococcus pneumoniae*.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding a polypeptide comprising a sequence selected from the group consisting of the sequences set out in the Sequence Listing, or a variant of any of these sequences.

In another particularly preferred embodiment of the invention there is a novel protein from *Streptococcus pneumoniae* comprising an amino acid sequence selected from the group consisting of the sequences set out in the Sequence Listing, or a variant of any of these sequences.

In accordance with another aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the *Streptococcus pneumoniae* 0100993 strain contained in the deposited strain.

A further aspect of the invention there are provided isolated nucleic acid molecules encoding a polypeptide of the invention, particularly *Streptococcus pneumoniae* polypeptide, and including mRNAs, cDNAs, genomic DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

In accordance with another aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization. Among the particularly preferred embodiments of the invention are naturally occurring allelic variants of a polypeptide of the invention and polypeptides encoded thereby.

Another aspect of the invention there are provided novel polypeptides of *Streptococcus pneumoniae* as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

Among the particularly preferred embodiments of the invention are variants of the polypeptides of the invention encoded by naturally occurring alleles of their genes.

In a preferred embodiment of the invention there are provided methods for producing the aforementioned polypeptides.

5 In accordance with yet another aspect of the invention, there are provided inhibitors to such polypeptides, useful as antibacterial agents, including, for example, antibodies.

 In accordance with certain preferred embodiments of the invention, there are provided products, compositions and methods for assessing expression of the polypeptides and polynucleotides of the invention, treating disease, for example, otitis media, conjunctivitis,
10 pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal fluid, assaying genetic variation, and administering a polypeptide or polynucleotide of the invention to an organism to raise an immunological response against a bacteria, especially a *Streptococcus pneumoniae* bacteria.

15 In accordance with certain preferred embodiments of this and other aspects of the invention there are provided polynucleotides that hybridize to a polynucleotide sequence of the invention, particularly under stringent conditions.

 In certain preferred embodiments of the invention there are provided antibodies against polypeptides of the invention.

20 In other embodiments of the invention there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit or activate an activity of a polypeptide or polynucleotide of the invention comprising: contacting a polypeptide or polynucleotide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the polypeptide or polynucleotide to
25 assess the binding to or other interaction with the compound, such binding or interaction being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide or polynucleotide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity of the polypeptide or polynucleotide by detecting the presence or absence of a signal generated from
30 the binding or interaction of the compound with the polypeptide or polynucleotide.

 In accordance with yet another aspect of the invention, there are provided agonists and antagonists of the polypeptides and polynucleotides of the invention, preferably bacteriostatic or bacteriocidal agonists and antagonists.

In a further aspect of the invention there are provided compositions comprising a polynucleotide or a polypeptide of the invention for administration to a cell or to a multicellular organism.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

GLOSSARY

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

10 "Host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990)). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence it is intended that the nucleotide sequence of the tested polynucleotide is identical to the

reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted
5 or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more
10 contiguous groups within the reference sequence. Analogously, by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence is intended that the test amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid. In other words, to obtain
15 a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy
20 terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a
25 polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA.
30 "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded

regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation,

hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

"Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

DESCRIPTION OF THE INVENTION

Each of polynucleotide and polypeptide sequences provided herein may be used in the discovery and development of antibacterial compounds. Upon expression of the sequences with the appropriate initiation and termination codons the encoded polypeptide

can be used as a target for the screening of antimicrobial drugs. Additionally, the DNA sequences encoding preferably the amino terminal regions of the encoded protein or the Shine-Delgarno region can be used to construct antisense sequences to control the expression of the coding sequence of interest. Furthermore, many of the sequences disclosed herein also provide regions upstream and downstream from the encoding sequence. These sequences are useful as a source of regulatory elements for the control of bacterial gene expression. Such sequences are conveniently isolated by restriction enzyme action or synthesized chemically and introduced, for example, into promoter identification strains. These strains contain a reporter structural gene sequence located downstream from a restriction site such that if an active promoter is inserted, the reporter gene will be expressed.

Although each of the sequences may be employed as described above, this invention also provides several means for identifying particularly useful target genes. The first of these approaches entails searching appropriate databases for sequence matches in related organisms. Thus, if a homologue exists, the Streptococcal-like form of this gene would likely play an analogous role. For example, a Streptococcal protein identified as homologous to a cell surface protein in another organism would be useful as a vaccine candidate. To the extent such homologies have been identified for the sequences disclosed herein they are reported along with the encoding sequence.

20 **ORF Gene Expression**

Recently techniques have become available to evaluate temporal gene expression in bacteria, particularly as it applies to viability under laboratory and infection conditions. A number of methods can be used to identify genes which are essential to survival *per se*, or essential to the establishment/maintenance of an infection. Identification of an ORF unknown by one of these methods yields additional information about its function and permits the selection of such an ORF for further development as a screening target. Briefly, these approaches include:

1) **Signature Tagged Mutagenesis (STM)**: This technique is described by Hensel et al., Science 269: 400-403(1995), the contents of which is incorporated by reference for background purposes. Signature tagged mutagenesis identifies genes necessary for the establishment/maintenance of infection in a given infection model.

The basis of the technique is the random mutagenesis of target organism by various means (e.g., transposons) such that unique DNA sequence tags are inserted in close proximity to the site of mutation. The tags from a mixed population of bacterial mutants

and bacteria recovered from an infected hosts are detected by amplification, radiolabeling and hybridisation analysis. Mutants attenuated in virulence are revealed by absence of the tag from the pool of bacteria recovered from infected hosts.

In *Streptococcus pneumoniae*, because the transposon system is less well
5 developed, a more efficient way of creating the tagged mutants is to use the insertion-
duplication mutagenesis technique as described by Morrison et al., J. Bacteriol. 159:870
(1984) the contents of which is incorporated by reference for background purposes.

2) **In Vivo Expression Technology (IVET):** This technique is described by
Camilli et al., Proc. Nat'l. Acad. Sci. USA. 91:2634-2638 (1994), the contents of which is
10 incorporated by reference for background purposes. IVET identifies genes up-regulated
during infection when compared to laboratory cultivation, implying an important role in
infection. ORF identified by this technique are implied to have a significant role in
infection establishment/maintenance.

In this technique random chromosomal fragments of target organism are cloned
15 upstream of a promoter-less recombinase gene in a plasmid vector. This construct is
introduced into the target organism which carries an antibiotic resistance gene flanked by
resolvase sites. Growth in the presence of the antibiotic removes from the population those
fragments cloned into the plasmid vector capable of supporting transcription of the
recombinase gene and therefore have caused loss of antibiotic resistance. The resistant pool
20 is introduced into a host and at various times after infection bacteria may be recovered and
assessed for the presence of antibiotic resistance. The chromosomal fragment carried by
each antibiotic sensitive bacterium should carry a promoter or portion of a gene normally
upregulated during infection. Sequencing upstream of the recombinase gene allows
identification of the up regulated gene.

25 3) **Differential display:** This technique is described by Chuang et al., J. Bacteriol. 175:2026-2036 (1993), the contents of which is incorporated by reference for
background purposes. This method identifies those genes which are expressed in an
organism by identifying mRNA present using randomly-primed RT-PCR. By comparing
pre-infection and post infection profiles, genes up and down regulated during infection can
30 be identified and the RT-PCR product sequenced and matched to ORF 'unknowns'.

4) Generation of conditional lethal mutants by transposon mutagenesis:

This technique, described by de Lorenzo, V. *et al.*, Gene 123:17-24 (1993); Neuwald, A. F. *et al.*, Gene 125: 69-73(1993); and Takiff, H. E. *et al.*, J. Bacteriol. 174:1544-1553(1992), the contents of which is incorporated by reference for background purposes, identifies genes whose expression are essential for cell viability.

In this technique transposons carrying controllable promoters, which provide transcription outward from the transposon in one or both directions, are generated. Random insertion of these transposons into target organisms and subsequent isolation of insertion mutants in the presence of inducer of promoter activity ensures that insertions which separate promoter from coding region of a gene whose expression is essential for cell viability will be recovered. Subsequent replica plating in the absence of inducer identifies such insertions, since they fail to survive. Sequencing of the flanking regions of the transposon allows identification of site of insertion and identification of the gene disrupted. Close monitoring of the changes in cellular processes/morphology during growth in the absence of inducer yields information on likely function of the gene. Such monitoring could include flow cytometry (cell division, lysis, redox potential, DNA replication), incorporation of radiochemically labeled precursors into DNA, RNA, protein, lipid, peptidoglycan, monitoring reporter enzyme gene fusions which respond to known cellular stresses.

5) Generation of conditional lethal mutants by chemical mutagenesis: This technique is described by Beckwith, J., Methods in Enzymology 204:

3-18(1991), the contents of which are incorporated herein by reference for background purposes. In this technique random chemical mutagenesis of target organism, growth at temperature other than physiological temperature (permissive temperature) and subsequent replica plating and growth at different temperature (e.g. 42°C to identify ts, 25°C to identify cs) are used to identify those isolates which now fail to grow (conditional mutants). As above close monitoring of the changes upon growth at the non-permissive temperature yields information on the function of the mutated gene. Complementation of conditional lethal mutation by library from target organism and sequencing of complementing gene allows matching with unknown ORF.

6) RT-PCR: *Streptococcus pneumoniae* messenger RNA is isolated from bacterial infected tissue e.g. 48 hour murine lung infections, and the amount of each mRNA species assessed by reverse transcription of the RNA sample primed with random hexanucleotides followed by PCR with gene specific primer pairs. The determination of the presence and

amount of a particular mRNA species by quantification of the resultant PCR product provides information on the bacterial genes which are transcribed in the infected tissue. Analysis of gene transcription can be carried out at different times of infection to gain a detailed knowledge of gene regulation in bacterial pathogenesis allowing for a clearer understanding of which gene products represent targets for screens for novel antibacterials. Because of the gene specific nature of the PCR primers employed it should be understood that the bacterial mRNA preparation need not be free of mammalian RNA. This allows the investigator to carry out a simple and quick RNA preparation from infected tissue to obtain bacterial mRNA species which are very short lived in the bacterium (in the order of 2 minute half-lives). Optimally the bacterial mRNA is prepared from infected murine lung tissue by mechanical disruption in the presence of TRIzol (GIBCO-BRL) for very short periods of time, subsequent processing according to the manufacturers of TRIzol reagent and DNAase treatment to remove contaminating DNA. Preferably the process is optimised by finding those conditions which give a maximum amount of *Streptococcus pneumoniae* 16S ribosomal RNA as detected by probing Northern blots with a suitably labelled sequence specific oligonucleotide probe. Typically a 5' dye labelled primer is used in each PCR primer pair in a PCR reaction which is terminated optimally between 8 and 25 cycles. The PCR products are separated on 6% polyacrylamide gels with detection and quantification using GeneScanner (manufactured by ABI).

Each of these techniques may have advantages or disadvantage depending on the particular application. The skilled artisan would choose the approach that is the most relevant with the particular end use in mind. For example, some genes might be recognized as essential for infection but in reality are only necessary for the initiation of infection and so their products would represent relatively unattractive targets for antibacterials developed to cure established and chronic infections.

Use of the of these technologies when applied to the ORFs of the present invention enables identification of bacterial proteins expressed during infection, inhibitors of which would have utility in anti-bacterial therapy.

The invention relates to novel polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of *Streptococcus pneumoniae*, which is related by amino acid sequence homology to known polypeptide as set forth in Table 1. The invention relates especially to compounds having the nucleotide and amino acid sequence selected from the group consisting of the sequences set out

in the Sequence Listing, and to the nucleotide sequences of the DNA in the deposited strain and amino acid sequences encoded thereby.

Deposited materials

5 A deposit containing a *Streptococcus pneumoniae* 0100993 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (herein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 April 1996 and assigned deposit number 40794. The deposit was described as *Streptococcus pneumnoiae* 0100993 on deposit.

On 17 April 1996 a *Streptococcus pneumnoiae* 0100993 DNA library in *E. coli* was similarly deposited with the NCIMB and assigned deposit number 40800.

10 The *Streptococcus pneumoniae* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full length genes comprising the polynucleotides set forth in the Sequence Listing. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in
15 the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience
20 to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

A license may be required to make, use or sell the deposited strain, and compounds derived therefrom, and no such license is hereby granted.

Polypeptides

25 The polypeptides of the invention include the polypeptides set forth in the Sequence Listing (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those which have the biological activity of a polypeptide of the invention, and also those which have at least 50%, 60% or 70% identity to a polypeptide sequence selected from the group consisting of the sequences set out in the Sequence Listing or the relevant portion, preferably at
30 least 80% identity to a polypeptide sequence selected from the group consisting of the sequences set out in the Sequence Listing, and more preferably at least 90% similarity (more preferably at least 90% identity) to a polypeptide sequence selected from the group consisting of the sequences set out in the Sequence Listing, and still more preferably at least 95% similarity (still more preferably at least 95% identity) to a polypeptide sequence selected from the group consisting of

the sequences set out in the Sequence Listing, and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

The invention also includes polypeptides of the formula:



wherein, at the amino terminus, X is hydrogen, and at the carboxyl terminus, Y is hydrogen or a metal, R_1 and R_3 are any amino acid residue, n is an integer between 1 and 2000, and R_2 is an amino acid sequence of the invention, particularly an amino acid sequence selected from the group set forth in the Sequence Listing. In the formula above R_2 is oriented so that its amino
10 terminal residue is at the left, bound to R_1 , and its carboxy terminal residue is at the right, bound to R_3 . Any stretch of amino acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. In preferred embodiments n is an integer between 1 and 1000

A fragment is a variant polypeptide having an amino acid sequence that entirely is the
15 same as part but not all of the amino acid sequence of the aforementioned polypeptides. As with polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region, a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of
20 the amino acid sequence of the Sequence Listing, or of variants thereof, such as a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus. Degradation forms of the polypeptides of the invention in a host cell, particularly a *Streptococcus pneumoniae*, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix
25 and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Also preferred are biologically active fragments which are those fragments that mediate
30 activities of polypeptides of the invention, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal, especially in a human. Particularly preferred are fragments comprising receptors or domains of enzymes that confer a function essential for

viability of *Streptococcus pneumoniae* or the ability to initiate, or maintain cause disease in an individual, particularly a human.

5 Variants that are fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

In addition to the standard single and triple letter representations for amino acids, the term "X" or "Xaa" is also used. "X" and "Xaa" mean that any of the twenty naturally occurring amino acids may appear at such a designated position in the polypeptide sequence.

10 Polynucleotides

Another aspect of the invention relates to isolated polynucleotides that encode the polypeptides of the invention having a deduced amino acid sequence selected from the group consisting of the sequences in the Sequence Listing and polynucleotides closely related thereto and variants thereof.

15 Using the information provided herein, such as the polynucleotide sequences set out in the Sequence Listing, a polynucleotide of the invention encoding polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Streptococcus pneumoniae* 0100993 cells as starting material, followed by obtaining a full length clone. For example, to obtain a
20 polynucleotide sequence of the invention, such as a sequence set forth in the Sequence Listing, typically a library of clones of chromosomal DNA of *Streptococcus pneumoniae* 0100993 in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent conditions. By
25 sequencing the individual clones thus identified with sequencing primers designed from the original sequence it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently, such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY*
30 *MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Illustrative of the invention, the polynucleotides set out in the Sequence Listing were discovered in a DNA library derived from *Streptococcus pneumoniae* 0100993.

The DNA sequences set out in the Sequence Listing each contains at least one open reading frame encoding a protein having at least about the number of amino acid residues set forth in the Sequence Listing. The start and stop codons of each open reading frame (herein "ORF") DNA are the first three and the last three nucleotides of each polynucleotide set forth in the Sequence Listing.

Certain polynucleotides and polypeptides of the invention are structurally related to known proteins as set forth in Table 1. These proteins exhibit greatest homology to the homologue listed in Table 1 from among the known proteins.

The invention provides a polynucleotide sequence identical over its entire length to each coding sequence in the Sequence Listing. Also provided by the invention is the coding sequence for the mature polypeptide or a fragment thereof, by itself as well as the coding sequence for the mature polypeptide or a fragment in reading frame with other coding sequence, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence. The polynucleotide may also contain non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence which encode additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA tag (Wilson *et al.*, *Cell* 37: 767 (1984). Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The invention also includes polynucleotides of the formula:



wherein, at the 5' end of the molecule, X is hydrogen, and at the 3' end of the molecule, Y is hydrogen or a metal, R_1 and R_3 is any nucleic acid residue, n is an integer between 1 and 3000, and R_2 is a nucleic acid sequence of the invention, particularly a nucleic acid sequence selected from the group set forth in the Sequence Listing. In the polynucleotide formula above R_2 is oriented so that its 5' end residue is at the left, bound to R_1 , and its 3' end residue is at the right, bound to R_3 . Any stretch of nucleic acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. In a preferred embodiment n is an integer between 1 and 1000

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Streptococcus pneumoniae* having an amino acid sequence set out in the Sequence Listing. The term also encompasses
5 polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or an insertion sequence or editing) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode for variants of the polypeptide having the deduced amino acid sequence of the Sequence
10 Listing. Variants that are fragments of the polynucleotides of the invention may be used to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding polypeptide variants, that have the amino acid sequence of a polypeptide of the Sequence Listing in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or
15 added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of such polynucleotide.

Further preferred embodiments of the invention are polynucleotides that are at least 50%, 60% or 70% identical over their entire length to a polynucleotide encoding a polypeptide having the amino acid sequence set out in the Sequence Listing, and polynucleotides that are
20 complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding a polypeptide of the deposited strain and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred
25 polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

A preferred embodiment is an isolated polynucleotide comprising a polynucleotide
30 sequence selected from the group consisting of: a polynucleotide having at least a 50% identity to a polynucleotide encoding a polypeptide comprising the amino acid sequence of the Sequence Listing and obtained from a prokaryotic species other than *S. pneumoniae*; and a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 50% identical to the

amino acid sequence of the Sequence Listing and obtained from a prokaryotic species other than *S. pneumoniae*.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by the DNA of the Sequence Listing.

The invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein.

The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in the Sequence Listing under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence or a fragment thereof; and isolating said DNA sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers described elsewhere herein.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding a polypeptide and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to a polynucleotide set forth in the Sequence Listing. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of each gene that comprises or is comprised by a polynucleotide set forth in the Sequence Listing may be isolated by screening using a DNA

sequence provided in the Sequence Listing to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

5 The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays.

10 Polynucleotides of the invention that are oligonucleotides derived from the a polynucleotide or polypeptide sequence set forth in the Sequence Listing may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

15 The invention also provides polynucleotides that may encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case
20 *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

25 A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

30 In addition to the standard A, G, C, T/U representations for nucleic acid bases, the term "N" is also used. "N" means that any of the four DNA or RNA bases may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a base that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

 In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a

preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Vectors, host cells, expression

5 The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

10 For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed.,
15 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

 Representative examples of appropriate hosts include bacterial cells, such as
20 streptococci, staphylococci, enterococci *E. coli*, streptomyces and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

 A great variety of expression systems can be used to produce the polypeptides of the
25 invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof,
30 such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any

of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention is also related to the use of the polynucleotides of the invention for use as diagnostic reagents. Detection of such polynucleotides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of a disease. Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, infected with an organism comprising a gene of the invention may be detected at the nucleic acid level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from an infected individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification technique prior to analysis. RNA or cDNA may also be used in the same ways. Using amplification, characterization of the species and strain of prokaryote present in an individual, may be made by an analysis of the genotype of the prokaryote gene. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing amplified DNA to labeled polynucleotide sequences of the invention. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, *e.g.*, Myers *et al.*, *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be

revealed by nuclease protection assays, such as RNase and S1 protection or a chemical cleavage method. See, e.g., Cotton et al., *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401 (1985).

Cells carrying mutations or polymorphisms in the gene of the invention may also be
5 detected at the DNA level by a variety of techniques, to allow for serotyping, for example. For
example, RT-PCR can be used to detect mutations. It is particularly preferred to use RT-PCR
in conjunction with automated detection systems, such as, for example, GeneScan. RNA or
cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers
complementary to a nucleic acid encoding a polypeptide of the invention can be used to identify
10 and analyze mutations. These primers may be used for, among other things, amplifying a DNA
of the invention isolated from a sample derived from an individual. The primers may be used to
amplify the gene isolated from an infected individual such that the gene may then be subject to
various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA
sequence may be detected and used to diagnose infection and to serotype and/or classify the
15 infectious agent.

The invention further provides a process for diagnosing, disease, preferably bacterial
infections, more preferably infections by *Streptococcus pneumoniae*, and most preferably otitis
media, conjunctivitis, pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and
endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal
20 fluid, comprising determining from a sample derived from an individual a increased level of
expression of polynucleotide having the sequence of the Sequence Listing. Increased or
decreased expression of a polynucleotide of the invention can be measured using any one of
the methods well known in the art for the quantitation of polynucleotides, such as, for
example, amplification, PCR, RT-PCR, RNase protection, Northern blotting and other
25 hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-
expression of a polypeptide of the invention compared to normal control tissue samples may be
used to detect the presence of an infection, for example. Assay techniques that can be used to
determine levels of a protein, in a sample derived from a host are well-known to those of skill in
30 the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western
Blot analysis and ELISA assays.

Antibodies

The polypeptides of the invention or variants thereof, or cells expressing them can be
used as an immunogen to produce antibodies immunospecific for such polypeptides.

"Antibodies" as used herein includes monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

Antibodies generated against the polypeptides of the invention can be obtained by
5 administering the polypeptides or epitope-bearing fragments, analogues or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pg. 77-
10 96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies.

15 Alternatively phage display technology may be utilized to select antibody genes with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing recognition of a polypeptide of the invention or from naive libraries (McCafferty, J. *et al.*, (1990), *Nature* 348, 552-554; Marks, J. *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can
20 also be improved by chain shuffling (Clackson, T. *et al.*, (1991) *Nature* 352, 624-628).

If two antigen binding domains are present each domain may be directed against a different epitope - termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides to purify the polypeptides by affinity chromatography.

25 Thus, among others, antibodies against a polypeptide of the invention may be employed to treat infections, particularly bacterial infections and especially otitis media, conjunctivitis, pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal fluid.

Polypeptide variants include antigenically, epitopically or immunologically
30 equivalent variants that form a particular aspect of this invention. The term "antigenically equivalent derivative" as used herein encompasses a polypeptide or its equivalent which will be specifically recognized by certain antibodies which, when raised to the protein or polypeptide according to the invention, interfere with the immediate physical interaction between pathogen and mammalian host. The term "immunologically equivalent derivative"

as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host.

The polypeptide, such as an antigenically or immunologically equivalent derivative
5 or a fusion protein thereof is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple copies of the protein or
10 polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized"; where the complementarity determining region(s) of the
15 hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones, P. et al. (1986), *Nature* 321, 522-525 or Tempest et al., (1991) *Biotechnology* 9, 266-273.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into
20 muscles (Wolff et al., *Hum Mol Genet* 1992, 1:363, Manthorpe et al., *Hum. Gene Ther.* 1993:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., *J Biol Chem.* 1989: 264,16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS*, 1986:83,9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., *Science* 1989:243,375), particle bombardment (Tang et al., *Nature* 1992,
25 356:152, Eisenbraun et al., *DNA Cell Biol* 1993, 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., *PNAS* 1984:81,5849).

Antagonists and agonists - assays and molecules

Polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural
30 product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, e.g., Coligan et al., *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of a polypeptides or polynucleotides of the

invention, particularly those compounds that are bacteriostatic and/or bacteriocidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising a polypeptide of the invention and a
5 labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be an agonist or antagonist of a polypeptide of the invention. The ability of the candidate molecule to agonize or antagonize a polypeptide of the invention is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of a polypeptide of
10 the invention are most likely to be good antagonists. Molecules that bind well and increase the rate of product production from substrate are agonists. Detection of the rate or level of production of product from substrate may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric labeled substrate converted into product, a reporter gene that is responsive to changes in polynucleotide or
15 polypeptide activity, and binding assays known in the art.

Another example of an assay for antagonists of polypeptides of the invention is a competitive assay that combines any such polypeptide and a potential antagonist with a compound which binds such polypeptide, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. A polypeptide of the
20 invention can be labeled, such as by radioactivity or a colorimetric compound, such that the number of such polypeptide molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide or polypeptide of the invention and thereby inhibit or
25 extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing activities induced by a polypeptide of the invention, thereby preventing the action of such polypeptide by excluding it from binding.

Potential antagonists include a small molecule that binds to and occupies the binding
30 site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC

Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of a polypeptide of the invention.

Each of the DNA sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide or inhibitor of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive bacteria, to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block protein-mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian tyrosine kinases (Rosenshine *et al.*, *Infect. Immun.* 60:2211 (1992); to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial proteins that mediate tissue damage and; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

The antagonists and agonists of the invention may be employed, for instance, to inhibit and treat otitis media, conjunctivitis, pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal fluid.

Helicobacter pylori (herein *H. pylori*) bacteria infect the stomachs of over one-third of the world's population causing stomach cancer, ulcers, and gastritis (International Agency for Research on Cancer (1994) Schistosomes, Liver Flukes and *Helicobacter Pylori* (International Agency for Research on Cancer, Lyon, France; <http://www.uicc.ch/ecp/ecp2904.htm>). Moreover, the international Agency for Research on Cancer recently recognized a cause-and-effect relationship between *H. pylori* and gastric adenocarcinoma, classifying the bacterium as a Group I (definite) carcinogen. Preferred antimicrobial compounds of the invention found using screens provided by the invention, particularly broad-spectrum antibiotics, should be useful in the treatment of *H. pylori* infection. Such treatment should decrease the advent of *H. pylori*-induced cancers, such as gastrointestinal carcinoma. Such treatment should also cure gastric ulcers and gastritis.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with a polypeptide of the invention, or a fragment or variant thereof, adequate to
5 produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Streptococcus pneumoniae* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a
10 nucleic acid vector to direct expression of a polynucleotide or polypeptide of the invention, or a fragment or a variant thereof, for expressing such polynucleotide or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that
15 disease is already established within the individual or not. One way of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid.

A further aspect of the invention relates to an immunological composition which,
20 when introduced into an individual capable or having induced within it an immunological response, induces an immunological response in such individual to a polynucleotide of the invention or protein coded therefrom, wherein the composition comprises a recombinant polynucleotide or protein coded therefrom comprising DNA which codes for and expresses an antigen of said polynucleotide or protein coded therefrom. The immunological response
25 may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

A polypeptide of the invention or a fragment thereof may be fused with co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. Thus
30 fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Hemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilize the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the

sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof which have been shown to encode non-variable regions of bacterial cell surface proteins in DNA constructs used in such genetic immunization experiments in animal models of infection with *Streptococcus pneumoniae* will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Streptococcus pneumoniae* infection, in mammals, particularly humans.

The polypeptide may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused, e.g., by mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant protein of the invention together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for

enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

While the invention has been described with reference to certain protein, such as,
5 for example, those set forth in the Sequence Listing, it is to be understood that this covers fragments of the naturally occurring protein and similar proteins with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant protein.

Compositions, kits and administration

10 The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or their agonists or antagonists. The polypeptides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount
15 of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the
20 invention.

Polypeptides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous,
25 intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application
30 for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol

or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially *Streptococcus pneumoniae* wound infections.

Many orthopedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1µg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Each reference disclosed herein is incorporated by reference herein in its entirety. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety.

10 TABLES

Certain pertinent data for each of the polypeptides and polynucleotides set forth in the Sequence Listing are summarized in the following Table.

TABLE 1

Provided in this Table is the closest homologue of each polypeptide encoded by each ORF of the invention based on a comparison of the sequences of in the Sequence Listing with sequences available in the public domain (see the right hand column entitled "Desc"). Where no significant homologue was detected "unknown" appears in the column. Preferred polypeptides encoded by the ORFs of the invention, particularly full length proteins either obtained using such ORFs or encoded entirely by such ORFs, are ones that have a biological function of the homologue listed, among other functions. The analysis used to determine each homologue listed in Table 1 was either BlastP, BlastX or MPSearch, each of which is well known. In the left hand column the SEQ ID NO: of each DNA sequence in the Sequence Listing appears. In the center column, the SEQ ID NO: of each protein (polypeptide) sequence of each sequence in the Sequence Listing appears across from the DNA sequence which encodes it (which DNA sequence is in the left hand column). In some instances the DNA sequence encodes more than one protein sequence and so is listed more than once, each listing being shown next to the encoded protein sequence.

DNA	Protein	Desc
===	=====	=====
1	323	unknown
2	324	probable transposase (insertion sequence IS861) - Streptococcus agalactiae (strain COH1)
3	325	unknown

What is claimed is:

1. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
 - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising an amino acid sequence of the Sequence Listing;
 - (b) a polynucleotide having at least a 70% identity to a polynucleotide encoding a mature polypeptide expressed by the gene contained in the *Streptococcus pneumoniae* of the deposited strain that was sequenced to obtain a polynucleotide sequence of the Sequence Listing;
 - (c) a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 70% identical to an amino acid sequence of the Sequence Listing;
 - (d) a polynucleotide which is complementary to the polynucleotide of (a), (b) or (c);and
 - (e) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a), (b), (c) or (d).
2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.
3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.
4. The polynucleotide of Claim 2 comprising the nucleic acid sequence selected from the group consisting of the nucleic acid sequences set forth in the Sequence Listing.
5. The polynucleotide of Claim 2 which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequences set forth in the Sequence Listing.
6. A vector comprising the polynucleotide of Claim 1.
7. A host cell comprising the vector of Claim 6.
8. A process for producing a polypeptide comprising: expressing from the host cell of Claim 7 a polypeptide encoded by said DNA.
9. A process for producing a polypeptide or fragment comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide or fragment.
10. A polypeptide comprising an amino acid sequence which is at least 70% identical to an amino acid sequence selected from the group consisting of the amino acid sequences set forth in the Sequence Listing.
11. A polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequences set forth in the Sequence Listing.

12. An antibody against the polypeptide of claim 10.
13. An antagonist or agonist of the activity or expression of the polypeptide of claim 10.
14. A method for the treatment or prevention of disease of an individual comprising: administering to the individual a therapeutically effective amount of the polypeptide of claim 10.
15. A method for the treatment of an individual having need to inhibit a bacterial polypeptide comprising: administering to the individual a therapeutically effective amount of the antagonist of Claim 13.
16. A process for diagnosing a disease related to expression or activity of the polypeptide of claim 10 in an individual comprising:
- (a) determining a nucleic acid sequence encoding said polypeptide, and/or
 - (b) analyzing for the presence or amount of said polypeptide in a sample derived from the individual.
17. A method for identifying compounds which interact with and inhibit or activate an activity of the polypeptide of claim 10 comprising:
- contacting a composition comprising the polypeptide with the compound to be screened under conditions to permit interaction between the compound and the polypeptide to assess the interaction of a compound, such interaction being associated with a second component capable of providing a detectable signal in response to the interaction of the polypeptide with the compound;
 - and determining whether the compound interacts with and activates or inhibits an activity of the polypeptide by detecting the presence or absence of a signal generated from the interaction of the compound with the polypeptide.
18. A method for inducing an immunological response in a mammal which comprises inoculating the mammal with the polypeptide of claim 10, or a fragment or variant thereof, adequate to produce antibody and/or T cell immune response to protect said animal from disease.
19. A method of inducing immunological response in a mammal which comprises delivering a nucleic acid vector to direct expression of a polypeptide of claim 10, or fragment or a variant thereof, for expressing said polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibody and/ or T cell immune response to protect said animal from disease.

20. A polynucleotide selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, and 74.

21. A polypeptide selected from the group consisting of SEQ ID NO: 323, 324, 325, 326, 327, 328, 329, 340, 341, and 403.

5 22. The isolated polynucleotide of claim 1 wherein said nucleotide is selected from the group consisting of:

(a) a polynucleotide having at least a 90% identity to a polynucleotide encoding a polypeptide comprising the amino acid sequence of the Sequence Listing;

10 (b) a polynucleotide having at least a 90% identity to a polynucleotide encoding the same mature polypeptide expressed by the gene contained in the *Streptococcus pneumoniae* of the deposited strain that was sequenced to obtain a polynucleotide sequence of the Sequence Listing;

(c) a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of the Sequence Listing;

15 (d) a polynucleotide which is complementary to the polynucleotide of (a), (b) or (c); and

(e) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a), (b), (c) or (d).

23. The isolated polynucleotide of claim 1 selected from the group consisting of:

20 (a) a polynucleotide having at least a 95% identity to a polynucleotide encoding a polypeptide comprising the amino acid sequence of the Sequence Listing;

(b) a polynucleotide having at least a 95% identity to a polynucleotide encoding the same mature polypeptide expressed by the gene contained in the *Streptococcus pneumoniae* of the deposited strain that was sequenced to obtain a polynucleotide sequence of the Sequence Listing;

25 (c) a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 95% identical to the amino acid sequence of the Sequence Listing;

(d) a polynucleotide which is complementary to the polynucleotide of (a), (b) or (c); and

30 (e) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a), (b), (c) or (d).

24. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide having at least a 50% identity to a polynucleotide encoding a polypeptide comprising the amino acid sequence of the Sequence Listing and obtained from a prokaryotic species other than *S. pneumoniae*;

5 (b) a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 50% identical to the amino acid sequence of the Sequence Listing and obtained from a prokaryotic species other than *S. pneumoniae*; and

(c) a polynucleotide which is complementary to the polynucleotide of (a) or (b).

25. An isolated Streptococcal polypeptide having one of the amino acid sequences given in Table 1.

10 26. An isolated nucleic acid encoding one of the amino acid sequences of Claim 1 and nucleic acid sequences capable of hybridizing therewith under stringent conditions.

27. Recombinant vectors comprising the nucleic acid sequences of Claim 26 and host cells transformed or transfected therewith.

15 28. A method of identifying an antimicrobial compound comprising contacting candidate compounds with a polypeptide of Claim 1 and selecting those compounds capable of inhibiting the bioactivity of said polypeptide.

29. Antimicrobial compounds identified by the method of Claim 28.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14436

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/02, 21/00; C12N 15/63, 1/20, 1/21

US CL : 536/23.1, 25.3; 435/320.1, 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 25.3; 435/320.1, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JORDAN, et al. Promoter identification and expression analysis of Salmonella typhimurium and Escherichia coli nrdEF operons encoding one of two class I ribonucleotide reductases present in both bacteria. Molecular Microbiology. 1996, Vol. 19, No. 4, pages 777-790, see entire document.	1-9, 20, 22-24, 26, 27
Y	RAUCH et al. Transcriptional regulation of the Tn5276-located Lactococcus lactis sucrose operon and characterization of the sacA gene encoding sucrose-6-phosphate hydrolase. Gene. 1992, Vol. 121, pages 55-61, see entire document.	1-9, 20, 22-24, 26, 27



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 OCTOBER 1997

Date of mailing of the international search report

05 NOV 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14436

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FENOLI et al. Molecular basis of the optochin-sensitive phenotype of pneumococcus: characterization of the genes encoding the Fo complex of the Streptococcus pneumoniae and Streptococcus oralis H ⁺ -ATPases. Molecular Microbiology. 1994, Vol. 12, No. 4, pages 587-593, see entire document.	1-9, 20, 22-24, 26, 27
Y	TATUSOV et al. Metabolism and evolution of Haemophilus influenzae deduced from a whole-genome comparison with Escherichia coli. Current Biology. 1996, Vol. 6, No. 3, pages 279-291, see entire document.	1-9, 20, 22-24, 26, 27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14436

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-9, 20, 22-24, 26, 27, species 1-3
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

- The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/14436

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

MASPAR, EMBASE, MEDLINE, BIOSIS, CAPLUS, BIOTECHDS, APS, JAPIO, WPIDS, CA, CONFSCI, LIFESCI
search terms: inventor names, polynucleotide, streptococcus pneumoniae, vectors, prokaryotic, cells

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-9, 20, 22-24, 26 and 27, drawn to a polynucleotide.

Group II, claims 10, 11, 21 and 25, drawn to a polypeptide.

Group III, claim 12, drawn to an antibody.

Group IV, claim 13, drawn to antagonist.

Group V, claims 14, 18 and 19, drawn to a method of treatment using a polypeptide.

Group VI, claim 15, drawn to a method of treatment using an antibody.

Group VII, claims 16 and 17, drawn to a method of diagnosis.

Group VIII, claim 28, drawn to a method identifying an antimicrobial compound.

Group IX, claim 29, drawn to antimicrobial compounds.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Species 1 includes SEQ ID Nos. 1-10

Species 2 includes SEQ ID Nos. 18, 28, 68, 74

Species 3 includes SEQ ID Nos. 104, 106, 155, 165

Species 4-176 includes the SEQ ID Nos. (in groups of 4 sequences) 11-17, 19-27, 29-67, 69-73, 75-103, 105, 107-154, 156-164, 166-713.

The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: because the special technical feature for each group is not the same.

The special technical feature of Group I is considered to be the polynucleotide sequences that encode the polynucleotide sequences that encode the polypeptide and the vectors and host cells.

The special technical feature of Group II is considered to be the polypeptide.

The special technical feature of Group III is considered to be an antibody directed against the polypeptide.

The special technical feature of Group IV is considered to be an antagonist to the polypeptide.

The special technical feature of Group V is considered to be the method of treatment using a unique polypeptide.

The special technical feature of Group VI is considered to be the method of treatment using an antibody to the polypeptide.

The special technical feature of Group VII is considered to be the methods and steps of diagnosis using the polypeptide.

The special technical feature of Group VIII is considered to be the methods and steps of identifying antimicrobial compounds.

The special technical feature of Group IX is considered to be antimicrobial compounds.

Accordingly, Groups I-IX are not so linked by the same or corresponding special technical feature as to form a single general inventive concept.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the species are different structurally and/or functionally. The 713 sequences are each different encoding different polynucleotide (DNA or RNA sequences) or polypeptide (amino acid sequences).